CLAIMS

1. A lipolytic enzyme which is a variant of a parent lipase derived from *Humicola lanuginosa* strain DSM 4109 comprising amino acid substitutions E1E,D,A +G91G,A,S,T +N94N,D +D96D,G,F,W +E99E,K +G225G,R,K +G263Q,N +L264L,A,V +I265I,T,S +G266G,A,V,S,D,E +T267T,A,V +L269L,I,N,Q.

2. The lipolytic enzyme of claim 1 which further comprises SPIRR as a peptide extension at the N-terminal and/or AGGF or AGGFS as a peptide extension at the C-terminal.

3. The lipolytic enzyme of claim 1 which further comprises a substitution P256A, or W260H,C,Q.

4. The lipolytic enzyme of claim 1 which has phospholipase activity, hydrolytic activity on digalactosyl-diglyceride (DGDG), a lower activity towards a C_4-C_8 acyl bond in a triglyceride, or a lower ratio of activity towards a C_4-C_8 acyl bond in a triglyceride and a C_{16}-C_{20} acyl bond in a triglyceride

5. The lipolytic enzyme of claim 4 which has phospholipase activity and is selected from the following variants of the parent enzyme:

E1A +G91A +D96W +E99K +P256A +W260H +G263Q +L264A +I265T +G266D +T267A +L269N +270A +271G +272G +273F (+274S)
SPIRR +G91A +D96W +E99K +G263Q +L264A +I265T +G266D +T267A +L269N +270A +271G +272G +273F (+274S)
E1A +G91A +D96W +P256A +W260H +G263Q +L264A +I265T +G266D +T267A +L269N +270A +271G +272G +273F (+274S)
E1A +G91A +D96W +E99K +P256A +W260H +G263Q +L264A +I265T +G266D +T267A +L269N
G263Q +L264A +I265T +G266D +T267A

E1SPPCGRRP +E239C +Q249R +G263Q +L264A +I265T +G266D +T267A
E1A +G91A +D96W +E99K +P256A +W260H +G263Q +L264A +I265T +G266D +T267A +L269N +270A +271G +272G +273F (+274S)
E1A +G91A +D96W +E99K +E239C +Q249R +P256A +G263Q +L264A +I265T +G266D +T267A +L269N +270A +271G +272G +273F (+274S)
E1A +G91A +D96W +E99K +N248T +Q249R +W260Q +G263Q +L264A +I265T +G266D +T267A +L269N +270A +271G +272G +273F (+274S)
SPIRR +G91A +D96W +E99K +W260C +G263Q +L264A +I265T +G266D +T267A +L269N +270A +271G +272 +G273F (+274S)
SPIRR +G91A +D96W +E99K +G263Q +L264A +I265T +G266D +T267A +L269N +270A +271G +272G +273F (+274S)
E1A +G91A +D96W +E99K +G263Q +L264A +I265T +G266D +T267A +L269N +270A +271G +272G +273F (+274S)
E1A +G91A +D96W +E99K +P256A +W260H +G263Q +L264A +I265T +G266D +T267A +L269N +270A +271G +272G +273F (+274S)
SPIRR +D96W +E99K +G263Q +L264A +I265T +G266D +T267A +L269N +270A +271G +272G +273F (+274S)
SPIRR +G91A +D96W +G263Q +L264A +I265T +G266D +T267A +L269N +270A +271G +272G +273F (+274S)
E1A +G91A +D96W +E99K +P256A +W260H +G263Q +L264A +I265T +G266D +T267A +L269N
E1A +G91A +D96W +E99K +P256A +W260H +G263Q +L264A +I265T +G266D +T267A +L269N +270A +271G
E1A +G91A +D96W +E99K +P256A +W260H +G263Q +L264A +I265T +G266D +T267A +L269N +270A +271G +272G
E1A +D96W +E99K +P256A +W260H +G263Q +L264A +I265T +G266D +T267A +L269N +270A +271G +272G +273F (+274S)
G91A +D96W +E99K +G263Q +L264A +I265T +G266D +T267A +L269N +270A +271G +272G +273F (+274S)
D96W +G263Q +L264A +I265T +G266D +T267A

6. The lipolytic enzyme of claim 4 which has an increased ratio of triolein activity to tributyrin activity and is selected from the following variants of the parent enzyme:

G91A +D96W +E99K +G263Q +L264A +I265T +G266D +T267A +L269N +270A

+271G +272G +273F (+274S)
D96W +G263Q +L264A +I265T +G266D +T267A +L269N +A270 +G271 +G272 +F273 +S274.
G91A +D96W +E99K +G263Q +L264A +I265T +G266D +T267A +L269N +270A +271G +272G +273F +274S

7. The lipolytic enzyme of claim 4 which has hydrolytic activity towards digalactosyl diglyceride (DGDG) and is selected from the following variants of the parent enzyme:

D96W +G263Q +L264A +I265T +G266D +T267A
G263Q +L264A +I265T +G266D +T267A
D96W +G263Q +L264A +I265T +G266D +T267A +L269N +270AGGFS
G91A +D96W +E99K +G263Q +L264A +I265T +G266D +T267A +L269N +270AGGFS

8. A lipolytic enzyme which is a variant of a parent lipolytic enzyme having an alcohol binding site having a glycerol part with an sn2 position, which variant:

a) comprises an alteration which is an insertion, a deletion or a substitution of an amino acid residue, at a position which in a three-dimensional structure of the parent lipolytic enzyme and a substrate is within 10 Å of the C atom at the sn2 position of the glycerol part of a substrate triglyceride, and

b) has an altered activity on an ester bond in the substrate.

9. A lipolytic enzyme which is a variant of a parent lipolytic enzyme having a lid, which variant:

a) comprises an alteration which is an insertion, a deletion or a substitution of an amino acid residue in the lid,

b) has an altered activity on an ester bond in the substrate.

10. A lipolytic enzyme which is a variant of a parent lipolytic enzyme having an active site comprising an active His residue, which variant:

a) comprises an alteration which is an insertion, a deletion or a substitution of at least one amino acid residue at the C-terminal side of the active His residue,

b) has an altered activity on an ester bond in a substrate.

11. A lipolytic enzyme which is a variant of a parent lipolytic enzyme, which variant:

a) comprises an alteration which is an insertion, a deletion or a substitution of at least one amino acid within 10 amino acid residues of the C-terminal,

b) has an altered activity on an ester bond in a substrate.

12. A lipolytic enzyme which:

a) is a polypeptide having an amino acid sequence which has at least 80 % homology with a reference lipolytic enzyme of the *Humicola* family or the *Zygomycetes* family;

b) compared to said reference lipolytic enzyme comprises an amino acid alteration which is:

i) a substitution, deletion or insertion at a position corresponding to A20, Y21, G23, K24, N25, V63, R81, G82, R84, A257, W260, Y261, F262 or G266 in the *Humicola lanuginosa* DSM 4109 lipase;

ii) a substitution of an amino acid corresponding to C268 or L269 in said lipase;

iii) a substitution corresponding to V60G, D62E, L93K, L97Q, K98E,F, E99D, P256A, G263E,Q,R,F,N, L264A,C,P,F,G,V,I, I265L,N,F or T267A,Q,P,S,V,E in said lipase;

iv) an insertion corresponding to T267GS or T267GL in said lipase;

v) a peptide extension at the C-terminal which is A, P, MD, CP, AG, DG, AGG, PVGF, AGRF, PRGF, AGGF or AGGFS;

vi) a peptide extension at the C-terminal of 40-50 amino acids; or

vii) a truncation of 1, 2, 3, 4, 5 or 6 amino acids at the C-terminal; and

c) has an altered activity on an ester bond in a substrate compared with the reference lipolytic enzyme.

13. A lipolytic enzyme which:

a) is a polypeptide having an amino acid sequence which has at least 80 % homology with a reference enzyme which is the lysophospholipase from *Aspergillus foetidus,* the ferulic acid esterase from *Aspergillus niger,* the ferulic acid esterase from *Aspergillus tubigensis* or phospholipase A1 from *Aspergillus oryzae*,

b) compared to said reference enzyme comprises an amino acid alteration which is a substitution, deletion or insertion at a position corresponding to 20-25, 56-64, 81-85, 91-98, 255-257 or 259-269 in the *Humicola lanuginosa* lipase, and

c) has an altered activity on an ester bond in a substrate compared with the reference enzyme.

14. The lipolytic enzyme of claim 8 wherein the altered activity is a lower activity towards a C_4-C_8 acyl bond in a triglyceride, or a lower ratio of activity towards a C_4-C_8 acyl bond in a triglyceride and a C_{16}-C_{20} acyl bond in a triglyceride.

15. The lipolytic enzyme of claim 14 which comprises an amino acid alteration at a position corresponding to Y21, E56, D57, V60, G61, D62, R81, S83, R84, L259, Y261 or G266 in the *Humicola lanuginosa* lipase.

16. The lipolytic enzyme of claim 8 wherein the altered activity is a higher hydrolytic activity on a digalactosyl-diglyceride.

17. The lipolytic enzyme of claim 16 which comprises an amino acid alteration at a position corresponding to Y21, G23, N26, D57, D62, R81, S83, R84, S85, G266, T267 or L269 in the *Humicola lanuginosa* lipase, preferably comprising two or more such alterations, most preferably further comprising at least one alteration in the lid region

18. The lipolytic enzyme of claim 8 which comprises an alteration in the lid which is a substitution of a negatively charged amino acid residue with a neutral or positively charged amino acid residue, or a substitution of a neutral amino acid residue with a positively charged amino residue.

19. The lipolytic enzyme of claim 18 which comprises an alteration in the lid at a position corresponding to position G91, D96 and/or E99 in the *Humicola lanuginosa* lipase, preferably a substitution which is G91A, D96S,W,F or E99K.

20. A DNA sequence encoding the lipolytic enzyme of claim 1.

21. A vector comprising the DNA sequence of claim 20.

22. A transformed host cell harboring the DNA sequence of claim 20.

23. A method of producing the lipolytic enzyme of claim 1 comprising

a) cultivating the cell of claim 22 so as to express and preferably secrete the lipolytic enzyme, and

b) recovering the lipolytic enzyme.

24. A process for preparing a dough or a baked product prepared from the dough which comprises adding the lipolytic enzyme of claim 1 to the dough, wherein the lipolytic enzyme preferably has phospholipase and/or digalactosyl diglyceride activity.

25. A process for reducing the content of phospholipid in an edible oil, comprising treating the oil with the lipolytic enzyme of claim 4 which has phospholipase activity so as to hydrolyze a major part of the phospholipid, and separating an aqueous phase containing the hydrolyzed phospholipid from the oil.

26. A process for improving the filterability of an aqueous solution or slurry of carbohydrate origin which contains phospholipid, which process comprises treating the solution or slurry with the lipolytic enzyme of claim 4 which has phospholipase activity, wherein the solution or slurry preferably contains a starch hydrolysate, particularly a wheat starch hydrolysate.

27. A detergent composition comprising a surfactant and the lipolytic enzyme of claim 1, wherein the lipolytic enzyme preferably has a specificity for long-chain fatty acids corresponding to a ratio of SLU to LU above 3.

28. A method of enhancing the flavor of a food product containing milk fat, comprising treating the food product with the lipolytic enzyme of claim 1 so as to release free fatty acids, wherein the lipolytic enzyme preferably has a specificity for short-chain fatty acids corresponding to a ratio of SLU to LU below 0.5, more preferably below 0.2, e.g. below 0.1.

29. A method of producing a lipolytic enzyme variant comprising:

a) selecting a substrate and an ester bond of interest,

b) selecting a parent lipolytic enzyme having an alcohol binding site having a glycerol part with an sn2 position,

c) in the parent lipolytic enzyme selecting at least one amino acid residue which comprises at least one atom within 10 Å of the C atom at the sn2 position of the glycerol part of a substrate triglyceride in a three-dimensional structure of the parent lipolytic enzyme and the substrate,

d) making alterations each of which is an insertion, a deletion or a substitution of the amino acid residue,

e) optionally, making alterations each of which is an insertion, a deletion or a substitution of an amino acid residue at one or more positions other than b),

f) preparing the variant resulting from steps b-d,

g) testing the activity of the variant on the selected ester bond,

h) selecting a variant having an altered activity on the selected ester bond, and

i) producing the selected variant.

30. A method of producing a lipolytic enzyme variant comprising:

a) selecting a substrate and an ester bond of interest,

b) selecting a parent lipolytic enzyme having a structure comprising a catalytic triad consisting of an active Ser, an active Asp and an active His residue,

c) in the parent lipolytic enzyme selecting at least one amino acid residue comprising at least one atom belonging to a set E defined by the following steps:

i) aligning the structure of the lipolytic enzyme with *Rhizomucor miehei* lipase structure 4TGL comprising a catalytic triad and an inhibitor phosphorus atom (4TGL-inhP), so as to minimize the sum of squares of deviation between atoms of the catalytic triads of the two structures,

ii) defining a set A consisting of atoms of the lipolytic enzyme inside a sphere of radius 18 Å with center at 4TGL-inhP,

iii) forming a first plane defined by 4TGL-inhP, the Cα atom of the active Ser residue of the parent lipolytic enzyme, and the Cα atom of the active Asp residue of the parent lipolytic enzyme and defining a set B as a subset of set A consisting of atoms on the same side of the first plane as the Cα atom of the active His residue of the parent lipolytic enzyme,

iv) forming a second plane defined by 4TGL-inhP, the Cα atom of the active Ser residue of the parent lipolytic enzyme, and the Cα atom of the active His residue of the parent lipolytic enzyme and defining a set C as a subset of set A consisting of atoms on the opposite side of the second plane from the Cα atom of the active Asp residue of the parent lipolytic enzyme,

v) forming a set D consisting of atoms belonging to the union of sets B and C, and having a solvent accessibility of 15 or higher, and

vi) forming set E consisting of amino acid residues in the structure which comprise an atom belonging to set D or an atom belonging to the union of sets B and C and located less than 3.5 Å from an atom belonging to set D,

d) making alterations each of which is an insertion, a deletion or a substitution of the selected amino acid residues,

e) optionally, making alterations each of which is an insertion, a deletion or a substitution of an amino acid residue at one or more positions other than d),

f) preparing the variant resulting from steps d) - f), and

g) testing the activity of the variant on the selected ester bond,

h) selecting a variant having an altered activity on the selected ester bond, and

i) producing the selected variant.

31. A method of producing a lipolytic enzyme variant comprising:

a) selecting a substrate and an ester bond of interest,

b) selecting a parent lipolytic enzyme having an active site comprising an active His residue,

c) in the amino acid sequence of the parent lipolytic enzyme selecting at least one amino acid residue at the C-terminal side of the active His residue,

d) making alterations each of which is an insertion, a deletion or a substitution of the amino acid residue,

e) optionally, making alterations each of which is an insertion, a deletion or a substitution of an amino acid residue at one or more positions other than b),

f) preparing the variant resulting from steps b-d,

g) testing the activity of the variant on the selected ester bond,

h) selecting a variant having an altered activity on the selected ester bond, and

i) producing the selected variant.

32. A method of producing a lipolytic enzyme variant comprising:

a) selecting a substrate and an ester bond of interest,

b) selecting a parent lipolytic enzyme,

c) selecting at least one amino acid residue among 10 amino acid residues at the C-terminal,

d) making alterations each of which is an insertion, a deletion or a substitution of the selected amino acid residues,

e) optionally, making alterations each of which is an insertion, a deletion or a substitution of an amino acid residue at one or more positions other than c),

f) preparing the variant resulting from steps c) - e),

g) testing the activity of the variant on the selected ester bond,

h) selecting a variant having an altered activity on the selected ester bond, and

i) producing the selected variant.

33. A method of producing a lipolytic enzyme variant comprising:

a) selecting a substrate and an ester bond of interest,

b) selecting a parent lipolytic enzyme having a lid,

c) selecting at least one amino acid residue in the lid,

d) making alterations each of which is an insertion, a deletion or a substitution of the selected amino acid residues,

e) optionally, making alterations each of which is an insertion, a deletion or a substitution of an amino acid residue at one or more positions other than c),

f) preparing the variant resulting from steps c) - e),

g) testing the activity of the variant on the selected ester bond,

h) selecting a variant having an altered activity on the selected ester bond, and

i) producing the selected variant.

34. A method of producing a lipolytic enzyme variant comprising:

a) selecting a substrate and an ester bond of interest,

b) selecting a parent lipolytic enzyme from the *Humicola* family or the *Zygomycetes* family,

c) selecting at least one amino acid residue corresponding to any of amino acids 20-25, 56-64, 81-85 and 255-269 in the *Humicola lanuginosa* lipase

d) making alterations each of which is an insertion, a deletion or a substitution of the amino acid residue,

e) optionally, making alterations each of which is an insertion, a deletion or a substitution of an amino acid residue at one or more positions other than c),

f) preparing the variant resulting from steps b-e,

g) testing the activity of the variant on the ester bond in the substrate, and

h) selecting a variant having an altered activity on the ester bond.

35. The method of claim 31 wherein the altered activity is a lower activity towards a C_4-C_8 acyl bond in a triglyceride, or a lower ratio of activity towards a C_4-C_8 acyl bond in a triglyceride and a C_{16}-C_{20} acyl bond in a triglyceride.

36. The method of claim 37 wherein the parent lipolytic enzyme belongs to the *Humicola* family or the *Zygomycetes* family, preferably the lipase of *Humicola lanuginosa* strain DSM 4109, and the selected amino acid residues comprise an amino acid corresponding to Y21, E56, D57, V60, G61, D62, R81, S83, R84, L259, Y261 or G266 in the *Humicola lanuginosa* lipase.

37. The method of claim 31 wherein the altered activity is a higher hydrolytic activity on a digalactosyl-diglyceride.

38. The method of claim 39 wherein the parent lipolytic enzyme belongs to the *Humicola* family or the *Zygomycetes* family, preferably the lipase of *Humicola lanuginosa* strain DSM 4109, and the selected amino acid residues comprise an amino acid corresponding to 21, 23, 26, 57, 62, 81, 83, 84, 85, 266, 267 or 269 in the *Humicola lanuginosa* lipase.